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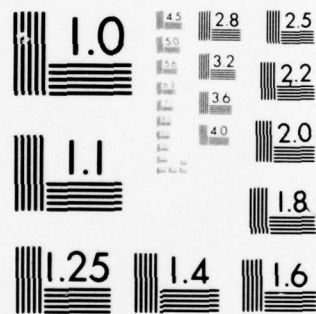
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IMMUNOLOGICAL STUDIES ON HEROIN ADDICTION

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Annual Report,

Jul 74 - Jun 75

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Chi-Tan Liu, ~~Ph.D.~~ and Frank L. Adler, ~~Ph.D.~~

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) → Hemagglutination-inhibition (HI) procedures for the detection and measurement of several abused drugs have been further developed and evaluated. Tests for morphine and for methadone have been applied to more than 30,000 urine specimens. The HI test for barbiturates is operational but requires further work toward increased sensitivity. An HI test for ecgonine has been shown to detect this drug and benzoylecgonine in urines containing 30 ng/ml or more of these drugs but suitable antisera have proven to be difficult to prepare. →		

Preliminary results suggest the feasibility of detecting LSD by HI.

→ The results of further studies on sporadic recurrent excretion of morphine are reported. Also presented are data which illustrate individual-specific responses to drug protein conjugates. Progress toward the use of immuno-adsorbants for concentration and purification of drugs in biological fluids is also reported.

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INTRODUCTION

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In continuation of our efforts to develop simple, sensitive and specific diagnostic test procedures for the detection of abused drugs in biological fluids, we have concentrated during the period covered by this report on two major subjects. One concerns the reported presence of antibodies reactive with morphine in the sera of heroin addicts, a phenomenon which is not only of interest for its potential diagnostic significance but also because of its possible bearing on drug tolerance or on the pathogenesis of so-called overdose reactions. The other major effort centered on the preparation of suitable reagents and the development of a hemagglutination-inhibition assay for the detection and measurement of lysergic acid diethylamide or its metabolites. Both major efforts have been demanding of time, and while we are pleased to report that good progress has been made in one we must admit to much remaining frustration in the other. In addition, we are presently concluding studies on the detection of methadone and methadone metabolites, and shall summarize our findings which we hope to publish shortly.

RESULTS1. The Immune Response to Repeated Administration of Morphine

The rather controversial subject of the presence of antibodies reactive with morphine in the sera of heroin addicts (Ryan *et al.*, 1972) appeared of sufficient interest to warrant thorough and systematic study. Since well documented clinical specimens are difficult or impossible to obtain from heroin users, an animal model suggested by the preliminary studies of others (Ringle and Herndon, 1973) was further developed and standardized by us. We developed techniques which allowed us to cause rabbits to develop predictable rises in the specific binding of morphine by their sera. The immunological mechanism(s) responsible for this increase in binding was documented physico-chemically by demonstrating that administration of the morphine by inappropriate schedules or in unsuitable amounts led to immunological unresponsiveness rather than demonstrable immunity, and serologically by appropriate tests for the specificity of the binding. The technical details of this work have been incorporated into two papers that have been submitted for publication. These two papers are submitted, in preprint form, as part of this report.

While we have not yet applied our findings from the animal model to human sera, we feel that much of the controversy in the literature stems from the poor binding of the antibody to morphine and the resulting difficulty in measuring the reaction. We have found equilibrium dialysis to be the method of choice for observing and measuring the antibody produced in rabbits. Conventional radioimmunoassay procedures are less suitable because the degree of binding is so highly dependent on concentration factors, and hemagglutination procedures proved to be entirely inadequate for the purpose at hand. Another facet of the work

yet to be explored more fully relates to the development of a high level of skin reactivity in some of the rabbits that display significant increases in the binding of morphine by their serum gamma globulin. While it seems most likely that the skin reactions seen are a manifestation of an acquired cellular immunity to morphine, one or the other of its metabolites, or to an in vivo conjugate of a tissue component and morphine or metabolite(s), this remains to be tested experimentally.

## 2. Recurrent Sporadic Excretion of "Morphine Equivalents"

It has previously been reported that "morphine equivalents", a term used to include morphine and antigenically related substances, appear sporadically after the first week of total abstinence from the drug in urine specimens from heroin addicted humans. The phenomenon appeared of sufficient interest to encourage the development of a suitable animal model (De Cato and Adler, 1973). Our yet limited experience with rhesus monkeys has not yet yielded satisfactory results, but we have been able to secure reasonably large amounts of urine from experimental mice by exploiting our preliminary finding that the phenomenon occurs with higher frequency in male than in female mice. We are presently in the process of analyzing pools of urine from such mice, some obtained three weeks or longer after last exposure of the animals to morphine. By the combined use of chromatographic and immunological procedures we hope to be able to relate the reactive material with one or more of the known metabolites of morphine.

We believe that recent work of others adds greatly to the interest in the phenomenon under discussion. We refer to the studies by Hughes (Brain Res. 88:295, 1975) and others which implicate endogenous substances, particularly rather small peptides, as pharmacologically active materials with morphine-like properties. It will be important to establish whether the peptides in question bear antigenic relationships to morphine and, if so, whether it may not be these endogenous materials that appear in urine many days or weeks after the excretion of the drug appears to have come to an end.

## 3. Studies on Methadone Metabolism and Excretion

In our recent paper (Liu and Adler, 1973) we reported that our hemagglutination-inhibition test for the detection of methadone in biological fluids could detect methadone in concentrations of about 10 ng/ml under practical test conditions. We also reported that we were able to detect methadone use for a period of about one week after last exposure to the drug. This conclusion was drawn from data obtained in the study of carefully collected and thoroughly documented 24-hour pooled specimens from one U.S. Armed Forces' member who had received

a single dose of 50 mg methadone during his hospitalization for withdrawal from heroin (made available to us by Maj. M. Robinson of WRAIR). Support came from less well documented specimens of individuals who had interrupted their methadone maintenance treatment for various periods of time.

We have completed a study of urine specimens obtained from babies born to mothers who are enrolled in methadone maintenance programs. The data obtained confirm those just mentioned. Taking the moment of birth as the last exposure to the drug, it was possible to show that methadone could be detected in urine specimens obtained during the first week of life. Since our antisera do not react with the major (cyclic) metabolites of methadone, namely 2-ethyl-5-methyl-3,3-diphenylpyrroline and 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine, it is apparent that either methadone itself or one of the minor metabolites that is reactive with our antibodies is excreted in measurable amounts for the 7-day period. We are presently applying chromatographic techniques to the "late" urine specimens in an attempt to identify the reactive material(s).

Of some interest is our failure to demonstrate prolonged excretion of methadone in animals of several species. We have treated rhesus monkeys, mice and rabbits with single or multiple injections of methadone, in doses that ranged widely, but have been unable to detect methadone beyond the third or fourth day after last injection. It may be that we are looking at differences in the metabolism of the drug when given orally or parenterally, or we may be observing species differences in the handling of the drug. Such differences might merely consist in a more efficient conversion of methadone into its cyclic and serologically unreactive metabolites in the experimental animals.

#### 4. Studies on Lysergic Acid and Lysergic Acid Diethylamide (LSD)

In our previous annual report we mentioned briefly that antisera had been prepared which reacted well with LSD and which could be used to measure LSD in solution by radioimmunoassay techniques with a sensitivity in the pg to ng/ml range. The antisera had been prepared by a method described in the studies of Taunton-Rigby et al. (Science 181:165, 1973) and our results closely agreed with theirs with respect to sensitivity and specificity of the radioimmunoassay applied to LSD and a series of chemically related compounds.

The HI test, only poorly developed at the time of our last report, has been developed to a point at which some but not all of the antisera prepared as just described are satisfactory reagents in a test that employs erythrocytes coated with a conjugate of human serum albumin and LSD. Applied to solutions of LSD, the HI test measures and detects the



drug in concentrations of 1-10 ng/ml. We have had satisfactory results with urine specimens from two experimental rhesus monkeys who had been injected once with LSD. The drug was detectable for 24-48 hours after injection but not beyond that time. Unfortunately, we have encountered considerable difficulties in applying the test to human urine specimens. Many such specimens from individuals who clearly had not taken LSD yielded "false positive" reactions, occasionally so intense that the presence of 100 ng/ml of LSD was suggested.

It should be stressed that the human urine specimens that give the "false positive" tests for LSD in the HI assay for LSD do not give false positive tests in the HI test procedures for methadone or morphine, nor do these specimens interfere in the radioimmunoassay for LSD. We may be the victims of a possible reaction between the human serum albumin component of the coating antigen (LSD-human serum albumin conjugate) and a component found in some but not other human urine specimens. A number of other possible explanations suggest themselves but do not appear worthy of discussion until further studies have been made.

It is our feeling that in due time the technical difficulties just discussed will be resolved. Once this has been accomplished that HI test might, as suggested by the data obtained from the study of monkey urines, be capable of detecting LSD use for 24-48 hours. However, in view of the relatively small amounts of this highly active drug that result in pharmacological activity, the urine concentrations even at the time of maximal excretion will be close to the limits of sensitivity of the test.

#### SUMMARY

In the body of this report and in the appended preprints we have reported on progress in our studies on the development of hemagglutination-inhibition tests for the detection of morphine, methadone and LSD. With regard to morphine and methadone, we have been primarily concerned with application and interpretation of the tests while the procedure applied to LSD is still in the developmental stage. Our investigations on the immune response to repeated administrations of morphine have been reported in detail because they contain considerable material that bears on the methodology of serological techniques and their application to the detection of drugs. Moreover, the possible significance of such immune responses in diagnostic procedures and in the understanding of drug tolerance and overdose reactions recommended the inclusion of the data in this report.

Publications

Beranek, J. T. and Adler, F. L. Binding of Morphine by Serum Globulins from Morphine Treated Rabbits. I. Induction and Measurement of Binding. Submitted for publication (preprints enclosed).

Beranek, J. T. and Adler, F. L. Binding of Morphine by Serum Globulins from Morphine Treated Rabbits. II. Antibody Nature of the Binding Globulins. Submitted for publication (preprints enclosed).

De Cato, L., Liu, C.-T. and Adler, F. L. Immunologic Studies on Drug Addiction. II. The Specificity of Antibodies Reactive with Methadone and its Metabolites. In preparation.

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